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**FATE OF WHITE FRINGETREE THROUGH THE INVASION WAVE OF
EMERALD ASH BORER AND ITS VARIATION IN RESISTANCE TO ATTACK**

A Thesis submitted in partial fulfillment of the
requirements for the degree of
Master of Science

by

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2020
Wright State University

WRIGHT STATE UNIVERSITY
GRADUATE SCHOOL

January 28, 2020

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY EMILY A. ELLISON ENTITLED "FATE OF WHITE FRINGETREE THROUGH THE INVASION WAVE OF EMERALD ASH BORER AND ITS VARIATION IN RESISTANCE TO ATTACK" BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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ABSTRACT

Ellison, Emily A. M.S., Department of Biological Sciences, Wright State University, 2020. FATE OF WHITE FRINGETREE THROUGH THE INVASION WAVE OF EMERALD ASH BORER AND ITS VARIATION IN RESISTANCE TO ATTACK

Ornamental white fringetrees in IL, IN, OH, and PA were reassessed in 2018 as a follow-up study to determine the fate of the white fringetree through the invasion wave of emerald ash borer. Attack rates decline by half from 2015-2018 and only 13% of trees were infested. Health of not reinfested trees in 2018 improved suggesting resilience against EAB attack whereas currently infested trees in 2018 displayed signs of declining health. Trees differed in their resistance to EAB attack and 41 additional ornamental and wild white fringetrees were studied in OH to determine if the anti-herbivory defense chemical, oleuropein, influenced EAB larval performance. Due to low attack rates and tolerance to EAB attack, white fringetrees are likely going to survive the invasion wave. Oleuropein concentrations were not correlated to larval performance, but oleuropein concentrations were higher in uninfested and ornamental trees suggesting genotypic and phenotypic variation among white fringetree genotypes.

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1 The fate of ornamental white fringetree through the invasion wave of emerald ash borer and implications for novel host use by this beetle

1.1 Introduction

The fate of a host tree in the face of an invasive insect pest is dependent on several biotic and abiotic factors, including the degree of resistance it expresses to the pest, the degree to which the host is being attacked, and environmental stressors. Several wood-boring beetles in the family Buprestidae are important invasive pests (Haack et al. 2002, Haack 2006, Coleman and Seybold 2008, Flint et al. 2013). These beetles can weaken and contribute to host mortality by girdling trees as larvae feed on vascular tissue. Buprestids tend to attack trees that have been compromised by pathogens, cankers, or environmental stress, such as drought (Goheen and Hansen 1993), but in some instances, can attack, weaken, and kill even healthy trees (Cappaert et al. 2005). Larger, stressed trees are known to be highly susceptible to wood and bark beetle attack and therefore are more likely to be killed than smaller (and younger), healthy trees. The gold spotted oak borer (*Agrilus coxalis* Waterhouse), the red oak borer (*Enaphalodes rufulus* Haldeman), and the mountain pine beetle (*Dendroctonus ponderosae* Hopkins) preferentially attack large, stressed and densely populated oak trees and pine trees, respectively, which leads to high mortality among these size classes (Goheen and Hansen 1993, Fierke et al. 2005a, Coleman et al. 2011). Beetle densities are also directly related to the degree to which a host tree may get attacked. For example, when mountain pine be

etle populations are low, only large, stressed pine trees get attacked, but as beetle populations increase, attack rates increase on all size classes (Goheen and Hansen 1993). Once host tree populations are depleted from beetle attack, pressure on surviving individuals is lowered as beetle populations decline in a local area.

Host range expansions can be achieved via ecological fitting when an organism uses inherent traits or characteristics to find and successfully utilize a novel host (Janzen 1985, Agosta 2006). A number of host range expansions have been documented among Buprestid beetles. Two lined chestnut borer (*Agrilus bilineatus* Weber) exemplifies this phenomenon because after its primary host, American chestnut, was decimated by chestnut blight, these beetles have primarily utilized stressed oak trees as a host (Dunn et al. 1986). Emerald ash borer (*Agrilus planipennis* Fairmaire) has also undergone a host range expansion. This invasive, Asian wood boring beetle is considered a specialist on ash trees (*Fraxinus* spp.) using Manchurian ash (*Fraxinus mandschurica* Rupr.) and a few other species as its primary ancestral hosts. Upon its arrival to North America in the 1990s (Siegert et al. 2014), EAB began to attack and decimate North American ash tree populations throughout the Eastern U.S. and southern Canada (Haack et al. 2002, McCullough and Roberts 2002). In 2014, it was discovered that EAB had been attacking another novel host in North America: white fringetree (*Chionanthus virginicus* L.) (Cipollini 2015), the first non-ash host confirmed for this beetle.

White fringetrees can be classified as trees or shrubs depending on the growth form of the individual; they grow up to 11 meters in height with an often multi-stemmed growth habit (USDA 2015). White fringetree is in the same family and closely related to ash trees (Oleaceae) (Wallander and Albert 2000) and are apparently physically and

chemically similar enough for EAB to utilize it as a host via ecological fitting (Cipollini and Peterson 2018). The native range of white fringetree extends as far north as New York, as far south as Florida and as far west as Texas, thus it grows alongside ash trees in its native habitats throughout North America, and they are planted as ornamentals within and outside of their native range in many of the same areas where ash trees grow and are planted (USDA 2015). White fringetree is mostly seed propagated and there are few recognized cultivars currently for sale, partly because of the difficulty in propagating them via cuttings (Russ 2010).

While the fate of North American ash trees in the face of EAB has been examined in a number of studies (Tanis and McCullough 2012, Spei and Kashian 2017), the fate of white fringetrees has only begun to be examined. When assessed in 2015, 26% of 178 white fringetrees growing in ornamental landscapes in Ohio, Illinois, Indiana, and Pennsylvania were found to be attacked by EAB, with most showing signs of canopy decline and a few trees dead due to EAB attack (Peterson and Cipollini 2017). At nearly all of the planted sites examined, at least some white fringetrees were found to be attacked, and trees had a higher probability of being attacked if they were larger, growing in denser populations, and had epicormic sprouting and canopy dieback (both symptoms of attack and indicators stress that may promote attack) (Peterson and Cipollini 2017). A dendrochronological study indicated that white fringetrees in southwestern Ohio first got attacked at about the same time as ash trees were attacked in the local area (Thiemann et al. 2016). Unfortunately, each of these studies was limited by being retrospective with no prior knowledge of the condition of trees assessed in each study and the exact timing of attack on them.

In this study, we reassessed the ornamental white fringetrees that were assessed in 2015 (Cipollini and Peterson 2017) to determine their fate through the invasion wave of EAB over the following three years. Their fate was determined by examining changes in infestation status as well as changes in the degree of canopy dieback and epicormic sprouting that could be observed since they were first assessed. We also examined how well factors such as tree size, canopy condition, and infestation status in 2015 could predict infestation status in 2018. Since beetle densities have largely declined in the areas encompassing our inventory of trees, we predicted that the majority of the trees would not be infested upon the 2018 reassessment. In turn, we predicted that tree health would generally stay the same or improve in these trees but would decline in those continually or newly infested. We also predicted that current year attack would be predicted by both past attack and tree health. Finally, we sought to expand the current map of white fringetree with signs of EAB attack in eastern North America. We predicted that the range of sites with evidence of EAB attack on white fringetree would continue to expand as the beetle has expanded its range.

1.2 METHODS

1.2.1 *White fringetree sites and reassessment*

We reexamined the same ornamental trees at the same sites used by Peterson and Cipollini (2017) in Illinois, Indiana, Ohio, and Pennsylvania (Table 1.1, Fig.1.1). Trees were located using our own tree inventory records along with the help of property managers. The majority of the fringetrees were open grown in well-manicured gardens, cemeteries, and arboretums; they showed evidence of periodic pruning and were often planted in mulch beds or mowed grass lawns, but none of them were treated with

insecticides, watered or fertilized as affirmed via personal communication with land managers. We saw no evidence that any trees in our study had been grafted, a technique that has generally not been used successfully for this species (University of Arkansas Cooperative Extension Factsheet 2006). Otherwise we had very limited information on the source of the trees planted in most of our sites. We reassessed a total of 166 of the original 178 trees due to the removal of 12 dead or unhealthy fringetrees from various sites since our initial survey.

Each fringetree was re-examined once in the summer (June, July, and August) of 2018 for canopy dieback, epicormic sprouting, and signs and symptoms of past or current emerald ash borer attack (Fig. 1.1). Every ornamental white fringetree in this study was examined via personal observations for EAB infestation, as we have done previously (Peterson and Cipollini 2017). The signs of EAB infestation include the presence of a D-shaped exit hole on stems or branches, feeding larvae or serpentine larval galleries beneath the bark containing larval frass. Symptoms of EAB attack on trees include circumferential swellings of stems or branches over feeding galleries, epicormic sprouting, and canopy dieback. If no external visible signs of attack were obvious, but there was suspicious bark swelling or other deformities, that particular area of the stem or branch was carefully debarked using a wood chisel to confirm gallery formation (Thiemann et al. 2016). Determining whether attack was new or old was based on inventory records from Peterson and Cipollini (2017) and on how deep the gallery was under the vascular cambium. If the gallery was near the newest layer of phloem tissue, more brown in color, had visible frass or feeding larvae, then it was considered new. If the gallery was under several layers of vascular tissue, appeared black in color, and had

no new frass or larvae, then it was considered old. A tree could have both new and old galleries; thus, we removed several layers of secondary xylem and phloem around suspicious areas to reveal galleries. Infestation status of each tree was categorized as newly infested, continuously infested, not reinfested, or never infested. Newly infested trees were trees that showed no signs of infestation by EAB in 2015 but were attacked at some point since then. Continuously infested trees were classified as infested in 2015 and again in 2018, whereas not reinfested trees were classified as infested in 2015, but with no signs of new infestation since then. Lastly, white fringetrees with no signs of infestation during either assessment were considered to be never infested. Canopies were visually assessed for dieback by one of the authors, which was quantified as a percentage of the canopy missing leaves. Each tree was placed into one of five categories corresponding to their percent dieback: 1 = 0% dieback; 2 = 25% dieback; 3 = 50% dieback; 4 = 75% dieback; and 5 = 100% dieback, based on the Gould et al. (2015) ash tree rating system. The presence or absence of epicormic sprouts, which grow from dormant buds beneath the bark of the tree that are activated and produce new branches when the tree is damaged or stressed, was recorded for each tree. Trees that were pruned for aesthetic reasons were documented in the event that the tree had lost epicormic sprouts recorded in 2015 due to pruning because trees typically do not lose epicormic sprouts that quickly, if at all. We did not re-measure the diameter of the largest stem (a metric recorded in the initial survey) due to difficulty in detecting the precise location of the previous measurement, and because these trees expand in diameter slowly.

1.2.2 Statistical Analysis

In our statistical analyses, we used data from both 2015 and 2018 to examine factors affecting the condition of trees in 2018, but otherwise analyzed these data in largely the same way as in Peterson and Cipollini (2017). Site was not included in our models in the original study or in this analysis due to vastly unequal replication of trees within sites. An ANOVA using PROC GLM and Tukey HSD Post Hoc test was used to examine variation in the diameter of the largest stem (measured in 2015), canopy dieback, and percent change in canopy dieback between 2015 and 2018 among infestation statuses, as determined in 2018. A Chi-Square test with PROC FREQ was used to compare the frequency of epicormic sprouting among infestation status categories as observed on trees in 2015 and 2018. We used binary regression with PROC LOGISTIC to determine relationships between current infestation status and the presence of epicormic sprouts (measured in 2015 and 2018), basal diameter of the largest stem (measured in 2015), crown dieback (measured in 2015 and 2018) and previous infestation status (measured in 2015). Stepwise model selection was used to select the best model with AIC. Data were analyzed using SAS (SAS Studio® Institute Inc., Cary, NC, 2018).

We published an initial distribution map of white fringetrees showing signs of attack by EAB in Peterson and Cipollini (2017), based on our observations through 2016. We sought to expand upon this map by adding newly discovered trees with signs of attack. This was not a systematic survey, and trees were not fully assessed as in our main study. In most cases, specific locations were visited to examine planted white fringetrees that were previously unknown to us and in some cases, trees were discovered opportunistically and assessed for signs of EAB attack. We confirmed any new infestations discovered through personal observations, or in two instances, relied on

photographic evidence from colleagues. The GPS coordinates of each previously and newly discovered site with at least one infested white fringetree was documented and mapped using QGIS software.

1.3 Results

Overall, we found that attack rates declined from 2015 to 2018. In 2015, 47 white fringetrees were infested out of 178 (Peterson and Cipollini 2017). Only 12 of the fringetrees were re-infested in 2018 and 10 were newly infested of 166 trees remaining in the study (Table 1.1). The majority of the trees were never infested (111) while 33 of the previously infested trees were not reinfested (Table 1.1). The 12 trees that were not reassessed in 2018 had been removed due to poor health, and in most cases, due to severe EAB infestation. One of the trees was removed in Yellow Springs, OH due to declining canopy health due to infestation by EAB. Two of the trees on Wright Patterson Airforce Base in Fairborn, OH were removed due to declining canopy health, but neither had confirmed EAB attack. Five trees were removed from Morton Arboretum in Lisle, IL due to declining canopy health: three of these were removed due to EAB infestation, and two trees were removed for unknown reasons. Lastly, two trees from the Chicago Botanic Gardens in Glencoe, IL were removed due to EAB infestation.

Average stem diameter varied among trees with different infestation statuses. On average, continuously attacked and not reinfested trees had the largest stem diameter and were more than 1.5 times larger than never infested trees ($F_{3,161}=8.82$, $P<0.001$) (Table 1.2). Newly attacked trees were slightly larger than never infested trees, but not significantly different than either continuously or not reinfested trees (Table 1.2).

The average percent canopy dieback in 2015 and 2018 varied significantly among trees with different infestation statuses, despite there being substantial variability in dieback estimates. In 2015, not reinfested trees, on average, had 2.6 times more canopy dieback than never infested trees ($F_{3,161} = 5.79$, $P = 0.0009$) (Table 1.2). In 2018, on average, newly and continuously infested trees had 3.8 times more canopy dieback than not reinfested and never infested trees, which did not differ from each other ($F_{3,161} = 3.02$, $P = 0.0313$) (Table 1.2).

Change in canopy dieback from 2015-2018 was significantly different between not reinfested versus newly, continuously, and never infested trees ($F_{3,161} = 5.39$, $P = 0.0015$) (Table 1.2). Canopy dieback worsened by 60% in newly infested trees followed by continuously (45%) and never (21%) infested trees (Table 1.2). Canopy dieback improved in not reinfested trees by 65% (Table 1.2).

We found variation in the frequency of epicormic sprouting in both 2015 ($\chi^2 = 36.84$, $P < 0.0001$) and 2018 ($\chi^2 = 15.36$, $P < 0.0001$) among infestation statuses (Table 1.3). Trees that had not been attacked in 2015 but that were classified as newly infested in 2018 showed significant increases in the frequency of epicormic sprouting. Trees that were infested in 2015 and continuously infested in 2018 had significantly more epicormic sprouting present than absent. The number of trees with epicormic sprouting declined by only 2 from 2015-2018, which was due to pruning, but presence of epicormic sprouting on trees remained significantly more common than its absence (Table 1.3). Not reinfested trees had significantly more epicormic sprouting present than absent in 2015, but this ratio declined in 2018. Never infested trees had significantly less epicormic sprouting

present than absent in both 2015 and 2018, with only 7 trees first acquiring sprouts between 2015 and 2018 (Table 1.3).

Canopy dieback in 2018, the infestation status in 2015, and epicormic sprouting in 2018 were significant predictors of infestation status in 2018 according to the stepwise regression model. For every 25% increase in canopy dieback in 2018, the trees were 9.6 times more likely to be infested ($\chi = 4.74$, $P = 0.029$). Fringetrees that were infested in 2015 were 3.8 times more likely to be infested in 2018 than those not previously infested ($\chi = 6.68$, $P = 0.009$). Fringetrees with epicormic sprouting in 2018 were 5.5 times more likely to be infested in 2018 ($\chi = 7.81$, $P = 0.005$). The other variables examined were not significant predictors of infestation in 2018.

Emerald ash borer attack on both ornamental and wild white fringetrees has been confirmed in five new states and at several new sites in states already known to hold attacked trees (Cipollini personal observation, Peterson personal observation, Ellison personal observation, Hoban personal communication) (Fig. 1.1, Table 1.1). Newly documented attacks on ornamental white fringetrees were found in gardens and arboretums in Toledo, OH, Cleveland, OH, West Portsmouth, OH, Newark, OH, Wooster, OH, Columbus, OH, Ann Arbor, MI, Bloomington, IN, Lexington, KY, Morgantown, WV, and on a private property in NC (Fig. 1.1). Infested trees in wild populations were found at the Latodami Nature Center in Wexford, PA, a private property near Jackson, OH, the Vinton Furnace State Experimental Forest in Oreton, OH, and on Bear Island in Potomac, MD (Fig. 1.1). Trees growing in wild populations typically grow at higher densities and appear to be attacked at higher rates than trees

planted at low density in ornamental landscapes, but these observations were not quantified in this study.

1.4 Discussion

We reexamined a group of ornamental white fringetrees first examined 3-4 years prior to this study to determine the fate of this novel host through the invasion wave of emerald ash borer. Based on our findings, it appears that this species will meet a better fate than most ash trees native to eastern North America. Concurring with our previous study (Peterson and Cipollini 2017), we found that the majority of the white fringetrees that we examined had not and were not being attacked by EAB. Moreover, of the trees that were previously attacked, most of them were not re-infested. This implies that beetle densities are decreasing in the areas encompassed by our survey as the peak of the invasion wave has moved away from these areas (http://www.emeraldashborer.info/timeline/by_county/index.html). Of trees that were attacked, tree stress (e.g., canopy dieback, epicormic sprouting, and previous infestation of the tree) was a good predictor (and sign) of current infestation. Accordingly, we should continue to see higher infestation rates on previously attacked or otherwise stressed trees in locations where there is sufficient beetle pressure, as seen in previous studies of EAB and with other Buprestids (Fierke et al. 2005b, Coleman et al. 2011, Tluczek et al. 2011). Previously attacked trees had higher odds of getting reinfested by EAB, but because beetle pressure in the areas encompassed by our survey has generally declined between 2015-2018, the number of reinfested trees in our survey was low. Trees that were reinfested or that were infested for the first time between 2015 and 2018 showed signs of declining health, such as increased canopy dieback and epicormic sprouting between

2015 to 2018, whereas trees not reinfested by EAB showed signs of recovery. The canopies of these trees were able to recover due to decreased stress on the tree and inherent wound healing and canopy regeneration. For those fringetrees experiencing new or continued attack, it can be inferred that their health will decline and that they will be even more prone to attack in the future, but should be able to recuperate if released from attack by the beetle.

As predicted, our results provide little evidence that white fringetrees are currently acting as a major reservoir for EAB (Cipollini and Peterson 2018). Oviposition and successful usage of white fringetree by EAB seems to transpire primarily when beetle densities and pressure are high and oviposition spillover occurs (Kaplan and Denno 2007). Because we surveyed areas where beetle densities and pressure were no longer high, further investigation in high pressure areas is required to support this hypothesis. Nonetheless, it appears that white fringetrees will not sustain a sufficiently viable population of EAB when growing in the kind of low density ornamental plantings that we surveyed to serve as a reservoir for EAB until ash tree populations rebound, as can be seen in other invasive pests that use alternative hosts until the availability of their preferred host increases (Lambert and Dudley 2014, Saeed et al. 2015). White fringetrees may not act as a reservoir for EAB in part because these trees are generally small and cannot support many individual beetles. However, wild populations may support more EAB because these plants grow more abundantly in the wild compared those planted ornamental sites (Cipollini personal observation), which could lead to increased use and higher beetle densities sustained by larger populations of white fringetrees.

Emerald ash borer primarily using white fringetree during times of high beetle pressure (Peterson and Cipollini 2017) suggests that these trees will continue to get attacked at low rates throughout its native range and continue to experience low mortality. Some white fringetrees, however, can be killed or at least damaged severely enough by EAB to warrant removal in ornamental landscapes. This was the case for about 7% of the trees that we examined between 2015 and 2018. Because white fringetrees varied from being never attacked at all to severely enough to be killed, significant variation in the susceptibility to EAB attack or its response to it apparently exists in this species. Every location that we examined had at least some infested white fringetrees, thus attack was not geographically structured, and location cannot explain the variation in infestation statuses among trees. The phenotypic and genotypic basis of this variation needs further study. According to the USDA plant database, there used to be a coastal variety of white fringetree given subspecies status (*Chionanthus virginicus* L. var. *maritimus* Pursh) that has since been reclassified simply as white fringetree; therefore, other scientists have recognized morphological variation within this species (USDA 2015). A few varieties of white fringetree are currently offered in the horticultural trade (Russ 2010), but they are uncommon and the majority of the trees are simply propagated from seed. If there is evidence of variation in plant resistance among white fringetrees, then those individuals or populations could be further investigated to understand potential mechanisms of resistance.

We found EAB attacking fringetrees in several new locations and in several new states since our initial observations in 2015 (Peterson and Cipollini 2017). If more white fringetrees were systematically surveyed where EAB is already present, we would likely

find additional trees under attack. As the invasion wave of EAB expands farther in North America, more white fringetrees will be exposed to attack. Importantly, the beetle is currently expanding in the native range of white fringetree where there are denser, wild populations and the tree is planted more commonly (USDA 2019). Emerald ash borer will have a continually increasing opportunity to infest white fringetrees in these locations. On the other hand, we have found that EAB is continuing to attack white fringetrees trees in areas, such as Ann Arbor, Michigan, close to where this beetle was first found in North America (USDA 2019). White fringetrees in this area were likely among the first individuals of this species that these beetles ever encountered. White fringetrees in Ann Arbor had poor health ratings and high mortality rates from EAB attack because of their lengthy exposure to attack and some have been severely pruned or removed (Cipollini personal observation). It is possible that EAB may be able to use white fringetree as a refuge to avoid biological control agents, such as *Tetrastichus planipennisi*, that has been released in the area to keep beetle densities below replacement (Duan et al. 2017). In controlled studies, however, *T. planipennisi* parasitized EAB larvae in white fringetree stems (Hoban et al. 2018, Olson and Rieske 2019). For white fringetree to serve as a reservoir for EAB, larvae will need to survive in densities high enough to overcome pressures from natural enemies such as *T. planipennisi*. One trait that may help white fringetrees tolerate EAB attack is due to having multiple stems; these trees are more resilient to attack than ash because they can lose several stems before being killed (Cipollini and Peterson personal observations). Conversely, ash trees have one main stem, and once that stem is effectively girdled by EAB, they are more likely to succumb to attack.

In conclusion, white fringetree is a novel host of EAB and will continue to get attacked throughout the U.S., especially as the EAB invasion encroaches on the native range of this species in the southeastern U.S. We anticipate finding variation in white fringetree resistance to EAB which could be from phenotypic or genotypic variation. Future research in plant resistance will help us better understand the variation in resilience that this tree has displayed over the course of three years in our initial and follow-up studies. While this plant has suffered low mortality and appears to be a poor reservoir for EAB in low density plantings, white fringetree may serve as a better refuge in locations where it is more abundant, which could then support sufficient numbers of EAB to maintain a local population. This is most likely to occur in the heart of the native range of white fringetree in the southeastern U.S. where this tree grows most densely in the wild.

Table 1.1. Study sites in Illinois, Indiana, Ohio, and Pennsylvania with GPS coordinates, total number of white fringetrees (*Chionanthus virginicus*) present in each site, and number of trees for each infestation status.

Site	State	GPS Coordinates	Number of trees	Infestation status			
				Newly	Continuously	Not reinfested	Never
Chicago Botanic Gardens	IL	42.1491° N, 87.7894° W	25	0	0	1	24
Cox Arboretum	OH	39.6554° N, 84.2243° W	18	4	3	1	10
Hershey Gardens	PA	40.2859° N, 76.6502° W	5	1	0	1	3
Indianapolis Museum of Art: Newfields	IN	39.8260° N, 86.1857° W	10	0	0	3	7
Morton Arboretum	IL	41.8164° N, 88.0549° W	14	1	0	5	8
Purdue University	IN	40.4237° N, 86.9212° W	20	1	4	1	14
Ferncliff Cemetery	OH	39.9242° N, 83.8088° W	4	0	0	3	1
Spring Grove Cemetery	OH	39.1743° N, 84.5250° W	36	0	1	11	24
Wilmington College	OH	39.4448° N, 83.8182° W	2	0	0	1	1
Woodland Cemetery	OH	39.7442° N, 84.1728° W	2	1	0	1	0

Wright Brother's Memorial	OH	39.4738°N, 84.0517° W	1	0	1	0	0
Wright Memorial Public Library	OH	39.7156° N, 84.1712° W	3	0	0	0	3
Wright-Patterson Air Force Base	OH	39.8137° N, 84.0537° W	8	0	0	1	7
Yellow Springs	OH	39.8064° N, 83.8869° W	18	2	3	4	9
Total:			166	10	12	33	111

Table 1.2. Mean tree diameter, percent canopy dieback in 2015 and 2018, and change in canopy dieback from 2015 to 2018 \pm standard deviation of ornamental white fringetrees (*Chionanthus virginicus*) across sites in IN, IL, OH, and PA. Different lowercase letters indicate significant differences via ANOVA with Tukey's HSD posthoc test $P < 0.05$).

Variable	Infestation status			
	Newly	Continuously	Not reinfested	Never
Avg diam (cm) 2015	6.61 \pm 2.08 ^{ab}	10.07 \pm 4.44 ^b	10.76 \pm 5.87 ^b	6.44 \pm 4.24 ^a
Avg % canopy dieback 2015	11.11 \pm 18.16 ^{ab}	15 \pm 19.54 ^{ab}	26.61 \pm 23.22 ^a	10.17 \pm 19.66 ^b
Avg % canopy dieback 2018	27.77 \pm 15.02 ^a	27.08 \pm 22.51 ^a	16.13 \pm 21.92 ^b	12.83 \pm 21.68 ^b
Change in canopy dieback from 2015-2018	-16.67 \pm 21.65 ^a	-12.08 \pm 27.42 ^a	10.48 \pm 25.64 ^b	-2.65 \pm 20.42 ^a

Table 1.3. Number of white fringetrees (*Chionanthus virginicus*) displaying the presence or absence of epicormic sprouting across United States field sites for each infestation status in 2015 and 2018.

	Frequency of epicormic sprouting by infestation status							
	Newly		Continuously		Not reinfested		Never	
	present	absent	present	absent	present	absent	present	absent
2015	4	5	12	0	23	8	33	80
2018	7	2	10	2	15	16	40	73

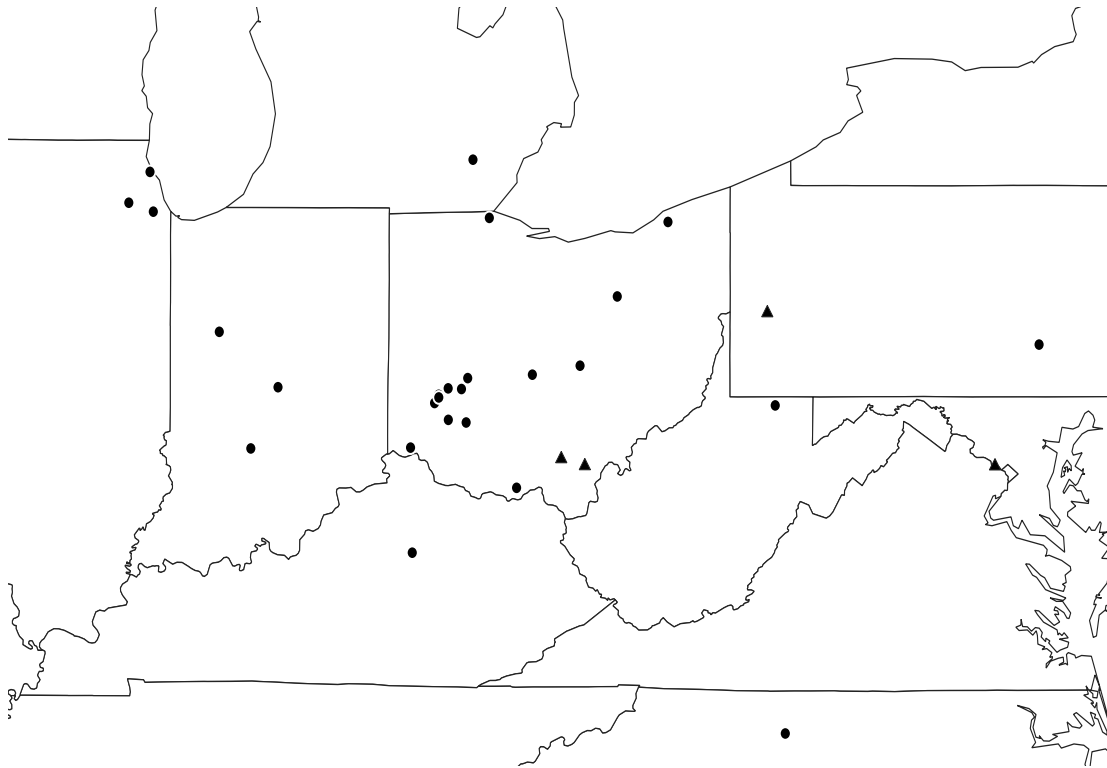


Figure 1.1. Locations where emerald ash borer (*Agrilus planipennis*) has been found attacking ornamental (circle) and wild (triangle) white fringetree (*Chionanthus virginicus*) populations in the eastern and midwestern United States.

2. Variation in EAB larval performance and oleuropein concentration within and among wild and ornamental white fringetrees

2.1 Introduction

Genetic variation within and among tree populations exists due to dispersal patterns, previous bottleneck effects, geographical location along with its corresponding environmental conditions, and silvicultural management of that species (Müller-Starck et al. 1992). The more widespread the species, the more genetically diverse that species will usually be, and, conversely, the closer the trees are to one another geographically, the more genetically similar they will usually be (Callahan 1962), unless environmental conditions greatly differ in a small area affecting the tree's micro and macro habitat, which can select for greater genetic diversity (Müller-Starck et al. 1992). Genotypic variation within and among populations of the same tree species can be assessed by analyzing their molecular, morphological, and phytochemical profiles as seen in Barbour et al.'s (2009) study on Eucalyptus trees (*Eucalyptus globulus*). These eucalyptus plants consists of genetically distinct geographic races that vary in leaf morphology and chemistry (Barbour et al. 2009). Trees that are geographically separated by physical barriers (i.e. a body of water, mountain range, land mass) tend to vary genetically population to population (Barbour et al. 2009) and can even be considered a different subspecies or races if the variation is great enough (Mallet 1995).

Because differing tree genotypes can express different traits, such as phenolic profiles and other secondary metabolites that can influence the quality of food for

herbivores, each genotype can differ in suitability for different herbivores which can lead to differences in herbivore communities (Awmack and Leather 2002, Holton et al. 2003). For example, leaf consumption varied 250-fold and growth varied 30-fold for gypsy moth larvae feeding on 12 *Populus* (Salicaceae) clones (Havill and Raffa 1999). Similarly, three out of four sawfly species (leaf folders, stem galls and petiole galls) varied in their densities on different arroyo willow (*Salix lasiolepis* Benthham) clones in the field (Fritz 1990). Differing sawfly densities on each tree indicates that there is variation in herbivore resistance among willow tree clones because more resistant trees had fewer sawflies present (Fritz 1990).

A suitable host plant is critical to herbivore fitness, and it is advantageous for female herbivores to lay their eggs and rear their offspring on the most suitable host possible. The “mother knows best hypothesis” suggests that female herbivores will preferentially lay eggs on host plants on which their larvae will have the best performance (Mayhew 2001, Rigsby et al. 2014). Thus, trees with differing genotypes may host different herbivores depending on herbivore choice, food quality, and suitability as a host plant. The mother knows best phenomenon is demonstrated by emerald ash borer (*Agrilus planipennis* Fairmaire), an invasive wood-boring beetle, that preferentially forages and oviposits on green ash (*F. pennsylvanica* Marshall), black ash (*F. nigra* Marshall), and white ash (*F. americana* L.) over blue ash (*F. quadrangulata* Michx.) due to inherent resistance mechanisms that blue ash are thought to have against EAB attack (Tanis and McCullough 2012, Tanis and McCullough 2015, Spei and Kashian 2017). Additionally, EAB acts in accordance with the mother knows best hypothesis in choosing native North American ash trees over its ancestral host, Manchurian ash (*Fraxinus*

mandshurica), for oviposition because larvae perform better on the susceptible North American trees than the resistant ancestral tree (Rigsby et al. 2014, 2016). Although EAB shows oviposition and feeding preferences among ash tree species, significant variation has not been observed in feeding or oviposition preference or larval performance within the same species of ash trees (Anulewicz et al. 2006, 2008, Peterson et al. 2015) indicating that there may be little variation among genotypes in EAB resistance within single ash species.

The inherent resistance mechanisms of blue and Manchurian ash could be due to the presence of certain phenolic compounds, such as oleuropein (Whitehill et al. 2012, Rigsby et al. 2016). Oleuropein is an anti-herbivory secondary metabolite (secoiriod glycoside) that is responsible for the bitter taste in olive fruits and is found in plants in the Oleaceae family, such as ash, olive (*Olea* spp.), and white fringetree (*Chionanthus virginicus* L.) (Soler-Rivas et al. 2000). A study found that larval performance and female oviposition preference of olive kernel moth (*Prays oleae* Bernard) varied between olive tree cultivars (*Olea europae* L.): females preferentially laid more eggs on nutritionally superior olive cultivars, which further supports the mother knows best hypothesis (Petrakis 2000). Moreover, oleuropein varies between olive cultivars (Petridis et al. 2012) and is the primary phenolic compound in trees in the family Oleaceae (Issaoui et al. 2012), which could be a factor in oviposition preference and larval performance. Olive trees are closely related to ash trees (*Fraxinus* spp. L.) and white fringetrees (Wallander and Albert 2000), and, thus, may share chemical resistance mechanisms to herbivores, such as EAB.

Emerald ash borer has expanded its host range from ash trees to the aforementioned close relative of ash trees: white fringetree, via ecological fitting (Cipollini 2015, Cipollini and Peterson 2018). White fringetree is a dioecious, insect-pollinated, multi-stemmed shrub or small tree native to the southeastern U.S. ranging from the east coast to as far westward as Texas (USDA 2015). These trees typically grow as scattered individuals in small populations in the wild and are also planted ornamentally inside and outside their native range. The physical separation and lack of gene flow between individuals in different populations may contribute to variation in a variety of traits among genotypes in this species. Emerald ash borer has been found to attack some white fringetrees but not others, even in the same geographical area and the same locations (Cipollini 2015, Cipollini and Rigsby 2015, Peterson and Cipollini 2018, Ellison et al. manuscript in revision). Several biotic and abiotic factors may contribute to variation within and among populations of this tree. It is unclear whether this variation in susceptibility is due to differing phenolic or volatile profiles of the trees that influence their detection by EAB, or if egg hatch, larval survivorship and larval performance vary between trees due to different chemical composition of the bark and vascular tissue. Undetected larvae and larval feeding can lead to false assumptions that trees are not being attacked when in reality, they are, but larvae are not surviving. Stress from previous EAB infestation or other factors might also play a role in the variation in susceptibility to EAB attack observed in white fringetree, which has also been observed in ash trees (McCullough et al. 2009, Tluczek et al. 2011). For example, Peterson and Cipollini (2017) found that white fringetrees that displayed signs of stress were more likely to have been attacked by EAB than healthy trees.

Recently, the fate of white fringetree through the invasion wave of EAB was studied and certain fringetrees were found to be completely unattacked, others somewhat resilient to EAB attack, while others were killed, suggesting that white fringetree genotypes vary in susceptibility and tolerance to EAB attack (Ellison et al. manuscript in revision). It is unknown what causes some white fringetrees to be resilient against attack and others more vulnerable, but the previously mentioned phenolic, oleuropein, may contribute to variation in resistance against EAB. Additionally, stress caused by previous infestation may affect susceptibility. In this study, we sought to characterize variation among white fringetree genotypes in resistance to EAB larvae and to determine if larval performance is correlated with the concentration of oleuropein in these trees. Additionally, we sought to characterize variation among wild and ornamental genotypes in resistance to EAB attack and whether previous infestation had any effect on susceptibility to attack. We expected to find varying larval performances and concentrations of oleuropein among sampled trees. We did not anticipate finding variation in larval performance between ornamental and wild white fringetrees because herbivore resistance mechanisms should be similar in both of these tree types and there is little evidence for selective breeding in ornamental white fringetrees for herbivore resistance. In contrast, we anticipated finding better larval performance on previously attacked trees based on the results of previous studies that found EAB preference for stressed trees (McCullough et al. 2009, Tluczek et al. 2011). We did not expect to find variation in oleuropein concentrations between wild and ornamental trees. Conversely, we expected to find higher concentrations of oleuropein in previously infested trees due to induced chemical responses to herbivory. Furthermore, we predicted to find a negative

correlation between larval performance and oleuropein concentrations due to the secondary metabolite's anti-herbivory properties.

2.2 Methods

2.2.1 Study sites, health assessment, and sample collection

Wild and ornamental fringetrees were assessed for EAB infestation and stem samples were taken from 11 sites throughout Ohio during a one-week time period in May 2019 (Figure 2.1). Trees were located using the Cipollini Lab white fringetree inventory records along with the help of property managers. The ornamental white fringetrees were grown in well-manicured parks and arboretums, and, therefore, were planted in mulched beds or mowed grass lawn areas for aesthetics, but none of them were treated with insecticides. Some showed signs of pruning. The wild populations were found in state parks or private land, but all were in protected deciduous forests and were also not treated with insecticides. Wild and ornamental white fringetrees are mostly seed propagated and there are few recognized cultivars currently for sale, partly because of the difficulty in propagating them via cuttings (Russ 2010). We saw no evidence that any trees in our study had been grafted, a technique that has generally not been used successfully for this species (University of Arkansas Cooperative Extension Factsheet 2006); therefore, each individual white fringetree is genetically unique and is considered its own genotype in this study. Each site was visited one time for the infestation and health assessment of the tree and collection of a stem and bark sample. A total of 40 trees were assessed and sampled from the 11 sites (Figure 2.1). White ash trees were also sampled alongside fringetrees at five sites throughout Ohio for a total of 11 trees (Figure 2.1). All study sites were documented on a map using QGIS Software (QGIS Development Team 2019).

The white fringetrees and ash trees were examined at their respective sites for evidence of EAB attack. Every tree in this study was examined via personal observations for EAB infestation. The signs of EAB infestation include the presence of a D-shaped exit hole on stems or branches, feeding larvae or serpentine larval galleries beneath the bark containing larval frass. Symptoms of EAB attack on trees include circumferential swellings of stems or branches over feeding galleries, epicormic sprouting, and canopy dieback. If no external visible signs of attack were obvious, but there was suspicious bark swelling or other deformity, that particular area of the stem or branch was debarked using a wood chisel to confirm gallery formation (Thiemann et al. 2016). Removing small amounts of bark to preserve the phloem can reveal new and old larval feeding galleries up to five years old (Thiemann et al. 2016). The evidence of attack was noted.

A healthy branch or main stem of approximately five centimeters in diameter from both fringe and ash trees was measured using a DBH tape and selected to be cut and transported back to Wright State University for a larval performance bioassay. A handsaw was used to cut a branch or the main stem if the tree was too young to have five-centimeter thick branches. The saw was disinfected with 100% ethanol before and after each use to avoid contamination. Cut stems were stored upright in five-gallon buckets with the ends submerged in water to facilitate water uptake. A disinfected utility knife was used to remove approximately one gram of bark tissue from each stem of white fringetree, which were dried on a laboratory bench before being sent to collaborators at Wittenberg University to determine oleuropein concentrations in the bark tissue.

2.2.2 Laboratory bioassay of larval performance

Our bioassay largely followed methods in Peterson et al. (2015). Cut stems were trimmed to approximately 40 cm in length, disinfected for 30 minutes by soaking them in a 10% commercial bleach solution, mosses and lichens were scrubbed off using a kitchen sponge to eliminate the potential growth of lingering mold, bacteria, or pathogens, and then rinsed thoroughly with distilled water. Stems were placed on a laboratory bench for several hours to thoroughly dry the bark surfaces before inoculating them with EAB eggs. EAB eggs were obtained from the USDA-APHIS-PPQ Biological Control Rearing Facility in Brighton, MI. Eggs were laid on coffee filters by adult females several days before their arrival to WSU where they were kept in a 25°C incubator with a 16:8 (L:D) cycle for one week. Each white fringetree and ash tree stem was inoculated with five EAB eggs. Coffee filters were cut so that one individual egg was on each piece of filter paper and then each egg was placed on each stem about five centimeters apart, leaving about 10 centimeters from both the top and bottom of the stem uninoculated. A one-centimeter wide piece of parafilm was placed over each of the filter papers to securely adhere the filter paper to the stem as in Peterson et al. (2015). Stems were then placed upright with their lower end submerged in DI water in five-gallon buckets and placed back in the incubator where the water was replaced and the stems rearranged on a weekly basis to minimize microenvironmental effects.

Observations of hatch rates were made one week after inoculation by unwrapping the parafilm and inspecting the egg under a microscope. A hole in the egg or coffee filter paper with frass around it indicated that the larva successfully hatched from the egg. A fine-point permanent marker was used to mark the area on the bark where the egg hatched. Successful hatches were recorded. Six weeks later in July 2019, stems were

debarked to quantify larval performance, which was determined by larval mass, gallery width, and survival. Bark was carefully shaved in thin sections from each stem with utility knives starting at each successful larval hatch site. Any feeding galleries that were produced by larvae were followed until they terminated. Larvae that were recovered were weighed to the nearest 0.1 mg and the widths of all feeding galleries were measured to the nearest 0.01 mm using a digital caliper at their terminus. Larval survivorship percentages on both white fringetree and ash tree stems were recorded and calculated as well.

2.2.3 HPLC analysis of oleuropein concentrations

Bark samples were dried on a laboratory bench for one week and then transported to Wittenberg University in Springfield, OH where collaborators ran a high-performance liquid chromatography (HPLC) analysis to determine the oleuropein concentrations in the bark tissue. Extractions were made via the Soxhlet extraction process: bark samples were hand shredded, weighed on an analytical balance, and poured into a cellulose thimble that was placed in the Soxhlet extractor. The extraction solvent was made from mixing 21mL of DI water with 104mL of ethanol and poured into a round bottom flask that was attached to the bottom of the extractor. A cold-water hose was attached to the top of the extractor, the heating mantle was turned on to heat the round bottom flask below, and the system was left to reflux for 2 hours and 40 minutes, which was determined based on previous tests of peak oleuropein concentrations during extraction (Al-Rimawi 2014). After running the extraction for the allotted time, the extraction liquid was removed from heat, and the liquid was stored in a test tube until it was run in the Agilent 1220 LC HPLC machine.

A 20 μ L HPLC syringe was used to inject the liquid extract into the HPLC machine. Each sample was run for 12 minutes with an 80% acetic acid buffer and 20% methanol solvent that linearly switched to 20% acetic acid buffer and 80% methanol solvent over the course of 6 minutes to obtain the peak area of oleuropein at 280nm (Malik and Bradford 2008). Oleuropein was found to elute at approximately 6 minutes based on a standard curve that was previously made. Each sample was run in the HPLC machine three times to average the peak oleuropein, which was then converted to oleuropein concentration in grams of oleuropein per 1g of tree tissue in relation to a standard curve.

2.2.4 Statistical analyses

Variation in larval performance and oleuropein concentrations among white fringetree and white ash tree genotypes was examined graphically (Figures 2, 3 & 4). An ANOVA was run to determine whether larval performance varied between the two species. Additionally, a Bartlett test was used to determine if the degree of variation in performance among different genotypes differed statistically between white fringetree and white ash.

A two-way ANOVA was used to test for significant variation in larval performance among tree types (ornamental/wild), infestation statuses (previously infested/not infested), and their interaction. Another two-way ANOVA was used to test for significant variation in oleuropein concentration among tree type, infestation status, and their interaction. Two-way ANOVAS were also used to determine variation in percent of successful EAB egg hatches, larval gallery establishment, and larval survivorship among tree type, infestation status, and their interaction.

A linear regression was used and the R^2 value was calculated to test if larval performance varied significantly with oleuropein concentrations of white fringetrees. Linear regressions were also used to test for associations between oleuropein concentrations and the percent of successful EAB egg hatches, larval gallery establishment, and larval survivorship. Statistical analyses were conducted in R (R Core Team 2019).

2.3 Results

2.3.1 Larval development and performance

We found variation among individual white fringetrees for percent of successful EAB egg hatches, established larval feeding galleries, and larval survivorship (Figures 2.2, 2.3 & 2.4). None of stages of larval development, such as the percent of successful egg hatches, established larval feeding galleries, or living larvae, were significantly different between tree type, infestation status, or the interaction between the variables. The percent of successful egg hatches did not vary between tree type ($F_{3, 37} = 1.87$, $P = 0.18$), infestation status ($F_{3, 37} = 0.003$, $P = 0.96$), or the interaction between the two variables ($F_{3, 37} = 0.18$, $P = 0.67$). The percent of established feeding galleries did not vary between tree type ($F_{3, 37} = 1.78$, $P = 0.19$), infestation status ($F_{3, 37} = 0.18$, $P = 0.67$), or the interaction between tree type and infestation status ($F_{3, 37} = 0.45$, $P = 0.50$). The percent of living larvae (survivorship) did not vary between tree type ($F_{3, 37} = 0.02$, $P = 0.89$), infestation status ($F_{3, 37} = 0.76$, $P = 0.39$) or the interaction between the two variables ($F_{3, 37} = 2.44$, $P = 0.13$). Although not significant, on average, infested wild white fringetrees tended to have higher hatch rates (5-14% more hatches) and larval

survivorship (17.5- 25% more surviving larvae) than other tree type and infestation status combinations.

We found a significant difference in larval performance (gallery width) between white fringetree and white ash reared larvae ($F_{1,117} = 37.93$, $P > 0.0001$) (Figure 2.5). On average, white ash tree reared larvae demonstrated better larval performance (3.45mm galleries) than white fringetree reared larvae (2.12mm galleries) (Figures 2.6 & 2.7). We found a slight trend that larval performance differed between infested wild and ornamental trees ($F_{3,119} = 6.73$, $P = 0.118$). Larvae had marginally better performance on wild trees than on ornamental trees. The interaction between tree type and infestation status had no effect on larval performance. We found no significant difference in relative variation in gallery width (larval performance) between white ash and white fringetree-reared larvae, but white fringetree-reared larvae had a 1.25 times larger range in larval performance than white ash reared larvae ($K^2 = 0.16$, $P = 0.68$).

2.3.2 *Oleuropein concentrations*

Concentrations of oleuropein varied between both tree type and infestation status. There was significantly more oleuropein in uninfested trees (0.12 g/sample) than in infested trees (0.08 g/sample) ($F_{3,119} = 12.91$, $P = 0.00047$) (Figure 2.8). Moreover, there was significantly more oleuropein in ornamental trees (0.11 g/sample) than in wild trees (0.08 g/sample) ($F_{3,119} = 6.55$, $P = 0.01$) (Figure 2.9). The interaction of tree type and infestation status did not significantly affect oleuropein concentrations ($F_{3,119} = 0.03$, $P = 0.87$), but, on average, uninfested ornamental white fringetrees tended to have the most oleuropein (0.13 g/sample) followed by uninfested wild (0.10 g/sample), infested ornamental (0.09 g/sample), and infested wild white fringetrees (0.06 g/sample).

Oleuropein concentrations ranged from 0.0046g/tissue sample to 0.303g/tissue sample among white fringetree genotypes (Figure 2.10).

2.3.3 Effect of oleuropein on larval development and larval performance

We found no significant correlation between oleuropein concentration and percent of successful EAB egg hatches ($F_{1,39} = 0.83$, $P = 0.37$), established larval feeding galleries ($F_{1,39} = 2.57$, $P = 0.12$) or surviving larvae ($F_{1,39} = 0.12$, $P = 0.73$). We also found no significant correlation between oleuropein concentrations and larval performance ($F_{1,39} = 0.02$, $P = 0.90$).

2.4 Discussion

We found variation in larval performance across our white fringetree genotypes ranging from no successful establishment of a larval feeding gallery to the development of fourth instar larvae, providing further evidence of variation among white fringetree genotypes in resistance to EAB attack. Furthermore, we found that larval performance, percent successful egg hatch, and larval survivorship tended to be better on infested wild white fringetrees than other combinations of tree type and infestation status. This trend can be explained by various contributing factors. Better overall larval performance on infested wild white fringetrees may be due to a weakening of defense mechanisms in trees in wild populations from biotic and abiotic factors, because they are not maintained or protected as are ornamental white fringetrees that are adequately managed. Additionally, previously infested trees are more likely to be reattacked by EAB (Peterson and Cipollini 2017). Emerald ash borer larvae also perform better on previously attacked white fringetrees (Peterson and Cipollini manuscript in review). White fringetree appears to vary in its resistance to EAB attack more substantially than ash trees (Ellison et al

manuscript in revision): larvae reach adulthood when reared on some white fringetrees, while other trees appear to kill most larvae (Cipollini and Rigsby 2015, Rutledge and Arango-Velez 2016, Peterson and Cipollini 2017, Peterson et al. manuscript in revision). Moreover, some white fringetrees get attacked while others do not, which suggests that white fringetrees vary in their initial susceptibility to attack as well (Ellison et al. manuscript in revision).

Although we did not see significant differences in variation between white ash and white fringetree in larval performance, the fate of ash trees is all the same: death due to EAB attack. Larvae are able to perform well enough to make it to adulthood on ash trees and kill the tree due to girdling it, and the majority of ash trees get attacked by EAB when the beetle encounters it. Even the worst performing larvae on a white ash trees still performed better than the majority of larvae on white fringetrees. No studies have been conducted on variation among ash tree genotypes within species and typically only one cultivar is used in ash tree studies (Villari et al. 2016). Since we sampled wild white ash trees from various locations in Ohio and saw slight variation in larval performance, we can attribute this variation to more genetic diversity seen in wild ash trees than in ornamental ash trees of the same cultivar or clone that are often used in studies (Cipollini et al. 2011, Whitehill et al. 2012).

In the field, it is possible that emerald ash borer did avoided white fringetree hosts with higher concentrations of oleuropein because it is anti-nutritive and an herbivore deterrent (Konno et al. 1999), which may explain why white fringetrees with higher concentrations of oleuropein remained uninfested. As predicted by the growth-differentiation balance hypothesis, wild white fringetrees likely have less oleuropein than

ornamental white fringetrees because they are allocating their energy and resources into growing and reproducing in less suitable conditions (e.g. more shade and competition) versus producing secondary metabolites, such as oleuropein, to defend against herbivores (Herms and Matteson 1992). Moreover, wild white fringetrees tend to grow in the understory of forests and, thus, are shaded, which has been found to decrease resistance to bark beetle herbivores (Waring and Pitman 1985, Christiansen et al. 1987).

Ornamental white fringetrees, on the other hand, can produce more secondary metabolites because they are grown in favorable conditions, such as high light and nutrient availability, and low competition, and, therefore, have sufficient resources to both grow and reproduce and to defend themselves (Herms and Matteson 1992).

Ornamental white fringetrees tended to vary more in their oleuropein concentrations than wild white fringetrees. One explanation for this increased variation is that ornamental white fringetrees are seed propagated in garden centers and nurseries, and the seeds come from various populations and geographical locations (Russ 2010, USDA 2015). White fringetrees are not generally cloned or successfully grafted (University of Arkansas Cooperative Extension Factsheet 2006), thus, ornamental white fringetrees should be more variable genetically since there is a broader gene pool than the geographically limited wild populations we studied.

Contradicting our hypothesis about finding little variation between infestation statuses in their oleuropein concentrations, we found that uninfested white fringetrees had higher concentrations of oleuropein than infested trees. Upon mechanical wounding, or in our study larval feeding, oleuropein is released from compartments in the cell where it was separated from its specific enzyme, beta glucosidase (Konno et al. 1999). Beta

glucosidase is the enzyme that cleaves off a glucose molecule from oleuropein converting it to a strong protein denaturant (Konno et al. 1999), which is antinutritive for herbivore pests (Spadafora et al. 2008). This enzymatic process is likely another reason we saw lower levels of oleuropein in infested trees because oleuropein was metabolized in the process of inducing defenses against feeding larvae (Konno et al. 1999, Spadafora et al. 2008). The reaction between beta glucosidase and oleuropein happens relatively quickly after wounding occurs, approximately 20 minutes (Spadafora et al. 2008), and may have converted oleuropein to an undetectable form in our analysis. Since cut stems were used in our study for the larval feeding bioassay, continued induced defenses or production of oleuropein was likely minimized; thus, oleuropein could have affected feeding larvae differently in living white fringetrees. Uninfested trees may have higher concentrations of oleuropein because there was no mechanical wounding to trigger the conversion of oleuropein to a protein denaturant. Rather, uninfested trees may have maintained their concentrations of constitutively produced oleuropein, which is why we saw higher concentrations of oleuropein in uninfested trees. The results of Spadafora et al.'s (2008) study of oleuropein concentrations in olive cultivars in reaction to mechanical wounding in olive fruits suggests that the concentration of oleuropein is not a good predictor of anti-herbivory defense ability. Instead, the efficiency of beta glucosidase in converting oleuropein into an antinutritive molecule should be measured when assessing anti-herbivory abilities of plants with oleuropein. Future research should address the efficiency of beta-glucosidase within and among EAB susceptible tree species to determine if the efficiency of beta-glucosidase is the reason for heightened resistance in certain host trees.

Additionally, other potential anti-herbivory defense chemicals found in Oleaceae trees that are thought to increase host tree resistance, such as pinolresinol and verbascoside (Eyles et al. 2007, Cipollini et al. 2011, Rigsby et al. 2015, Whitehill et al. 2014), should be analyzed to determine if they have an effect on larval performance in white fringetree and other susceptible Oleaceae trees. Bark and leaf tissues on white fringetrees should be tested comparatively as well for phenolic compounds because there is two to three times more oleuropein in bark than leaves on olive trees (Issaoui et al. 2012). These differing concentrations might play a role in host suitability for an herbivore pest and the efficiency of defense. Once we understand which compounds protect these trees against pests and how the physiology of the defense chemicals, we can better understand how defense chemicals influence their pests' ability to survive on them.

In conclusion, we detected substantial variation in EAB larval performance and in oleuropein concentrations among white fringetree genotypes. Both tree type and infestation status influenced these variables. Infested wild white fringetrees had lower concentrations of oleuropein compared to ornamental white fringetrees, which is likely why we saw a trend of better larval performance on wild white fringetrees than ornamentals. Additionally, uninfested white fringetrees had higher concentrations of oleuropein than previously infested trees. Emerald ash borer were likely deterred from selecting trees with higher oleuropein concentrations as a host, and infested white fringetrees used oleuropein as an induced response to defend against feeding larvae. Our study suggests that there is both genotypic and phenotypic variation among white fringetrees in EAB resistance.

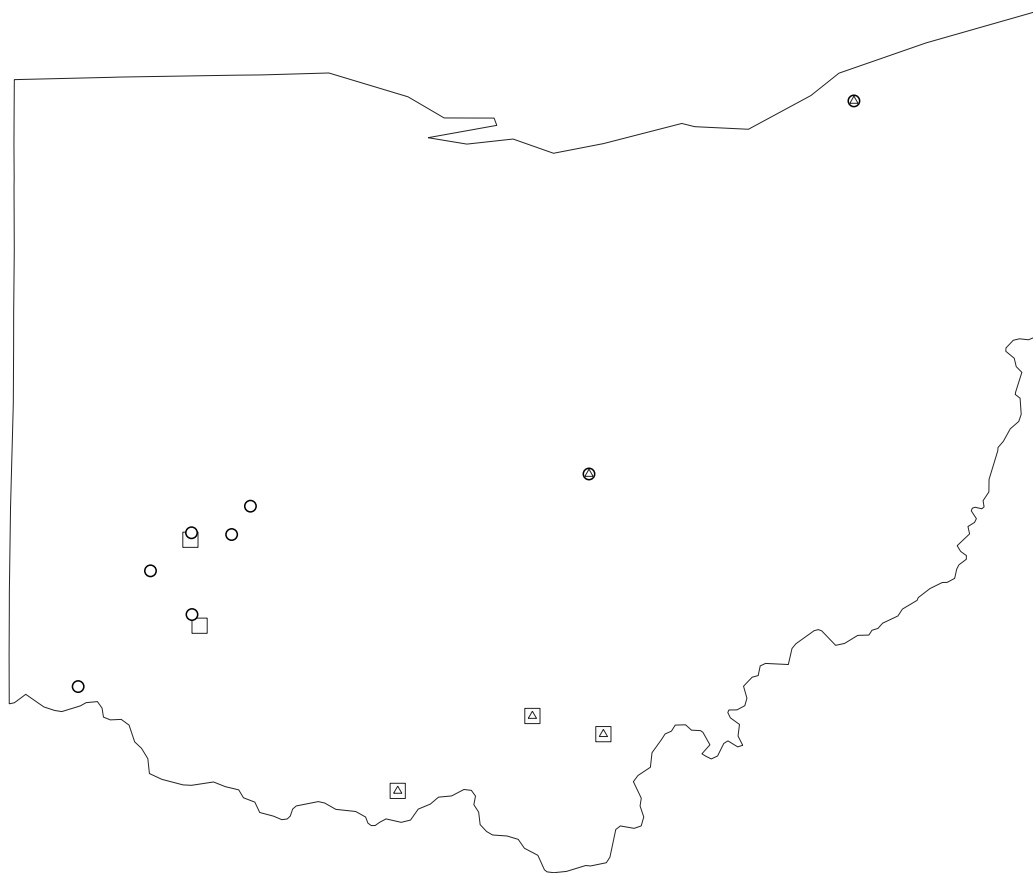


Figure 2.1. Locations of sampled ornamental white fringetrees (circle) (n=29), wild white fringetree (triangle) (n=12), and ash trees (square) (n=9) in Ohio.

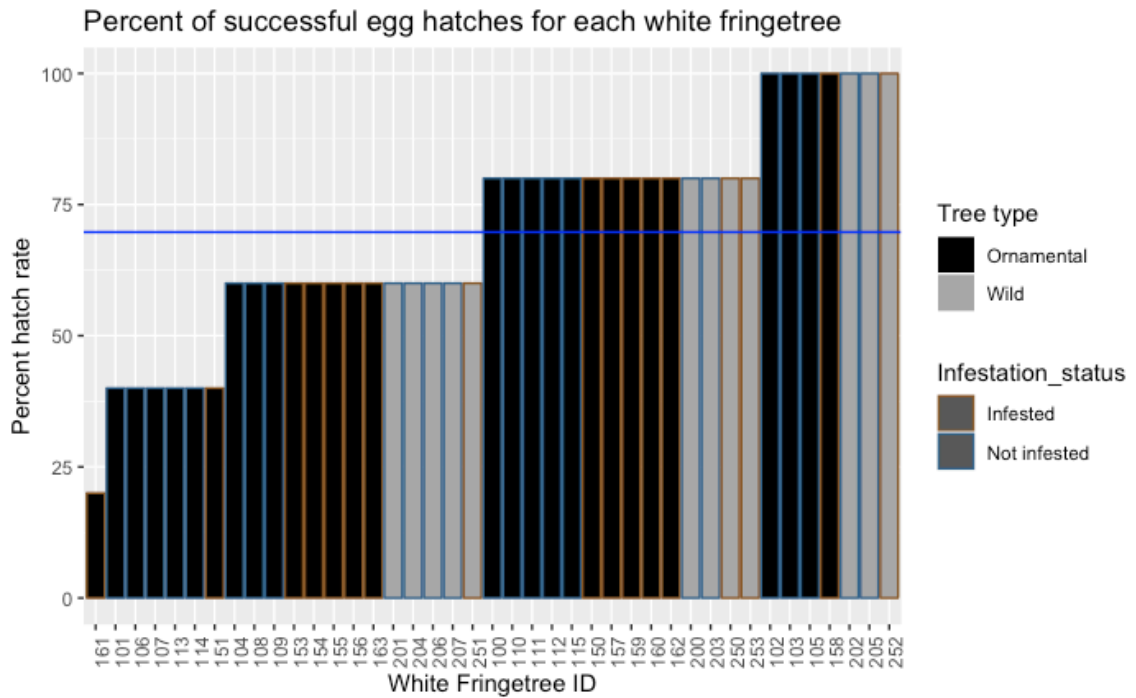


Figure 2.2. The percent of successful emerald ash borer (*Agrilus planipennis*) egg hatches for each white fringetree (*Chionanthus virginicus*) genotype with overall mean percent egg hatch line (69.75%), tree type, and infestation status.

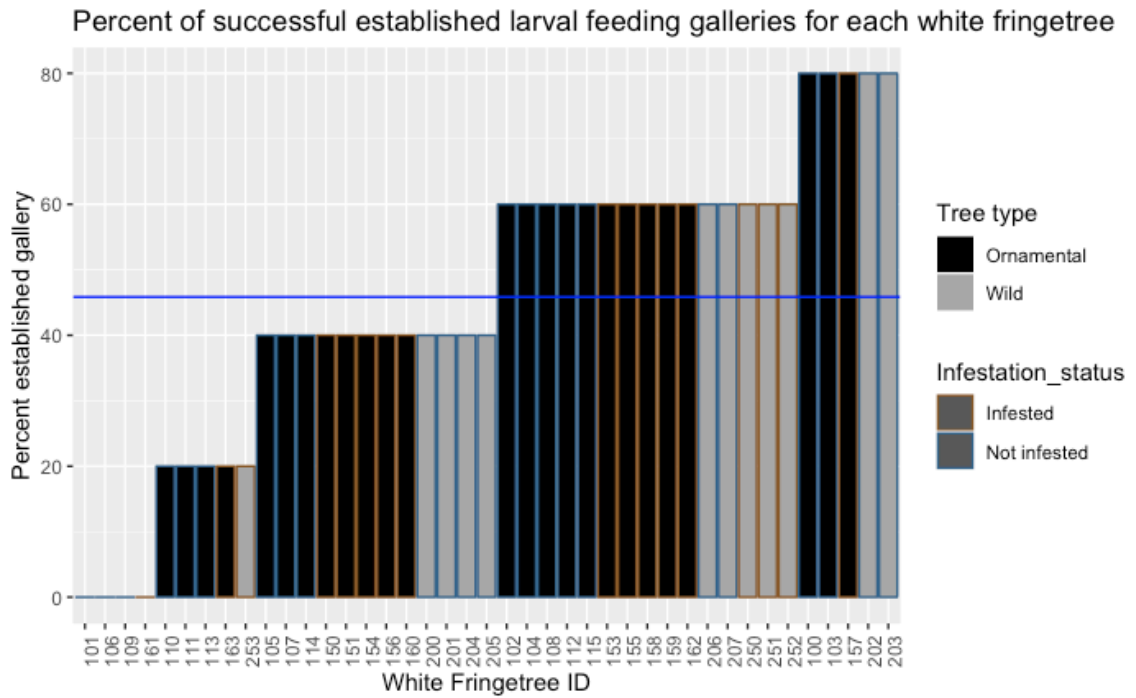


Figure 2.3. The percent of established emerald ash borer (*Agrilus planipennis*) larval feeding galleries for each white fringetree (*Chionanthus virginicus*) with overall mean percent of established larval feeding gallery line (45.85%), tree type, and infestation status.

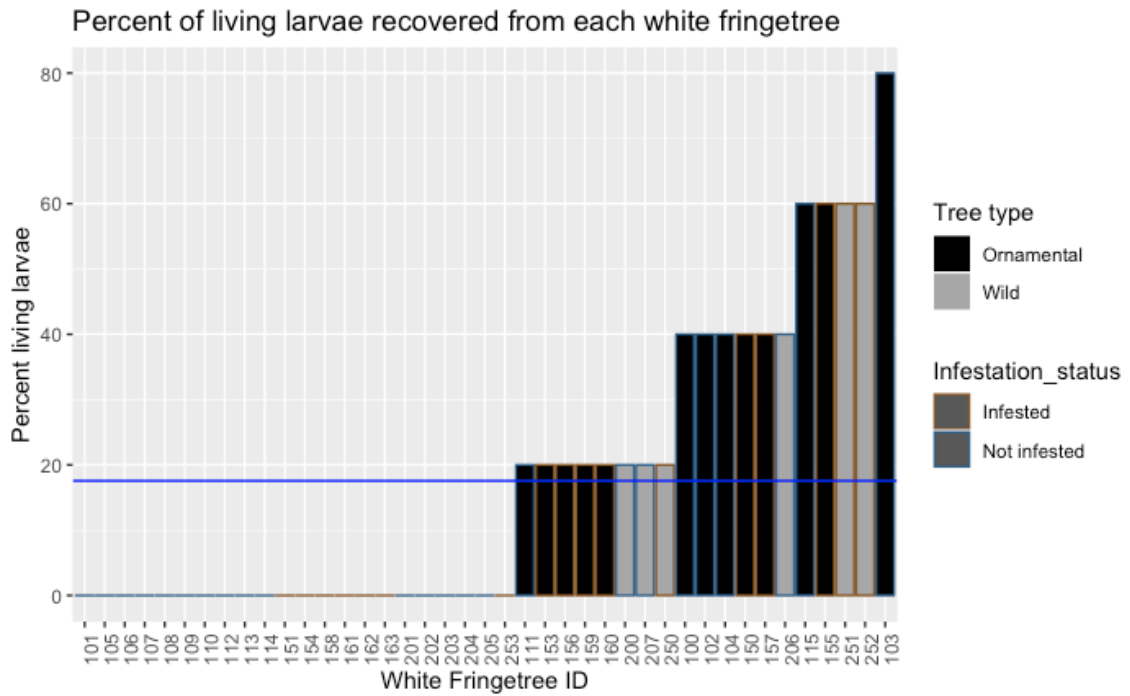


Figure 2.4. The percent of living emerald ash borer (*Agrilus planipennis*) larvae for each white fringetree (*Chionanthus virginicus*) with overall mean percent of living larvae line (17.56%), tree type, and infestation status.

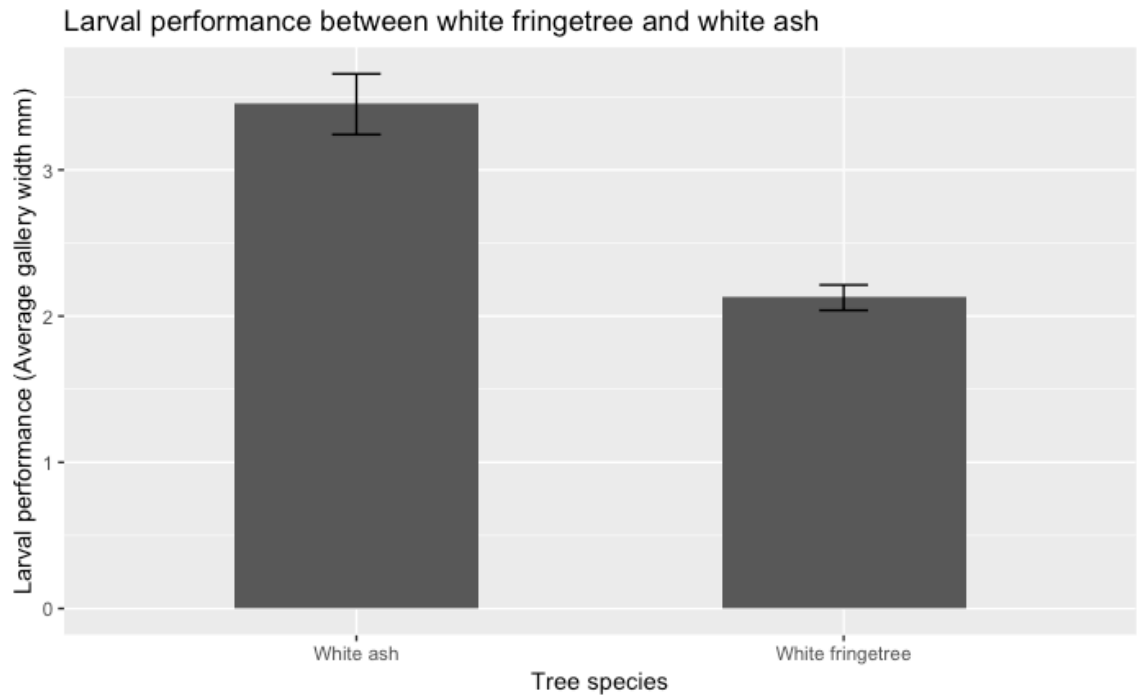


Figure 2.5. Average larval performance (gallery width) between white fringetree (*Chionanthus virginicus*) (2.12mm) and white ash (*Fraxinus americana*) (3.45mm) reared larvae.

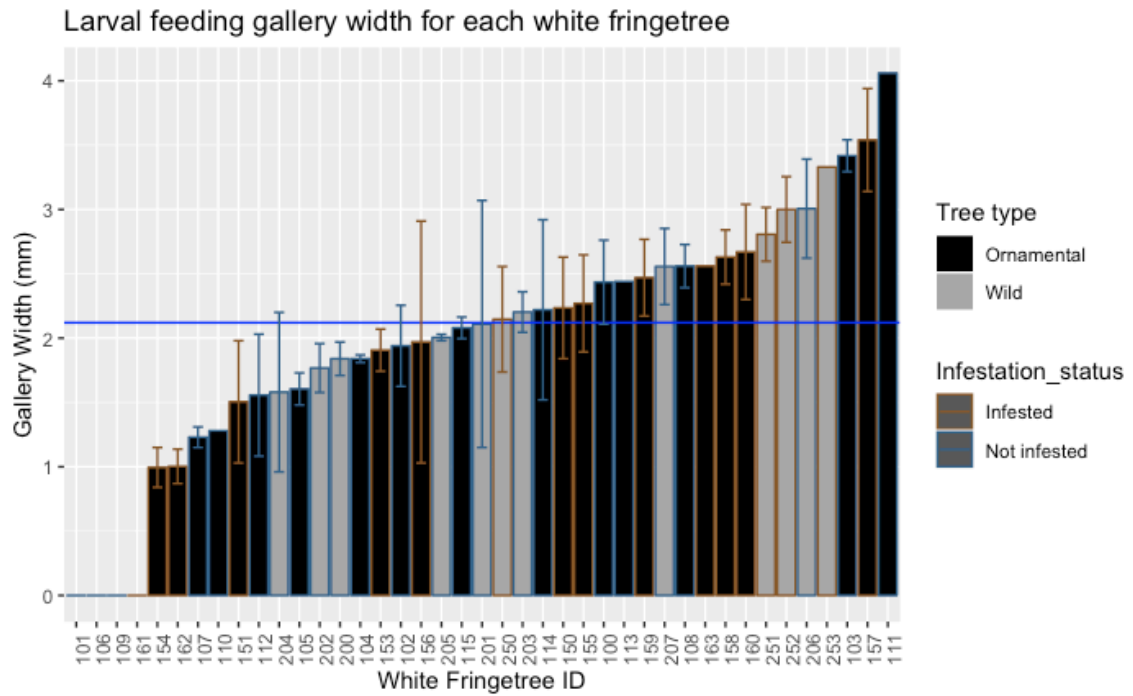


Figure 2.6. Gallery width in mm for each white fringetree (*Chionanthus virginicus*) genotype with standard error bars, tree type, and infestation status and overall mean gallery width line (2.25mm).

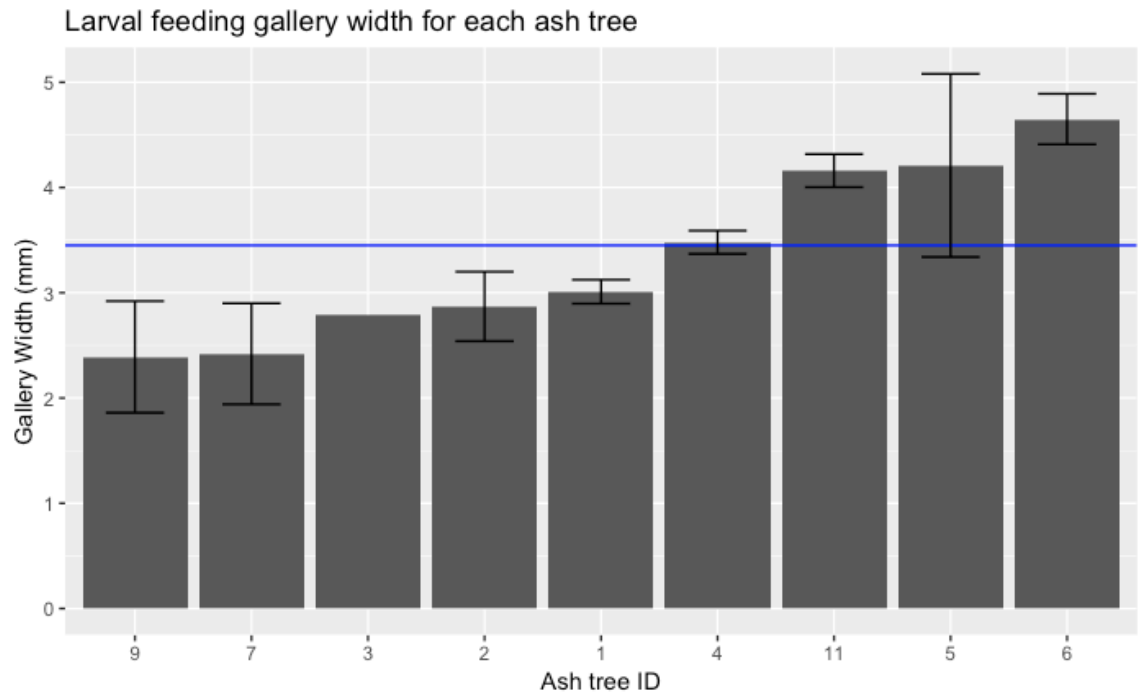


Figure 2.7. Larval performance (gallery width) for each white ash tree (*Fraxinus americana*) genotype with standard error bars and overall mean gallery width line (3.45mm).

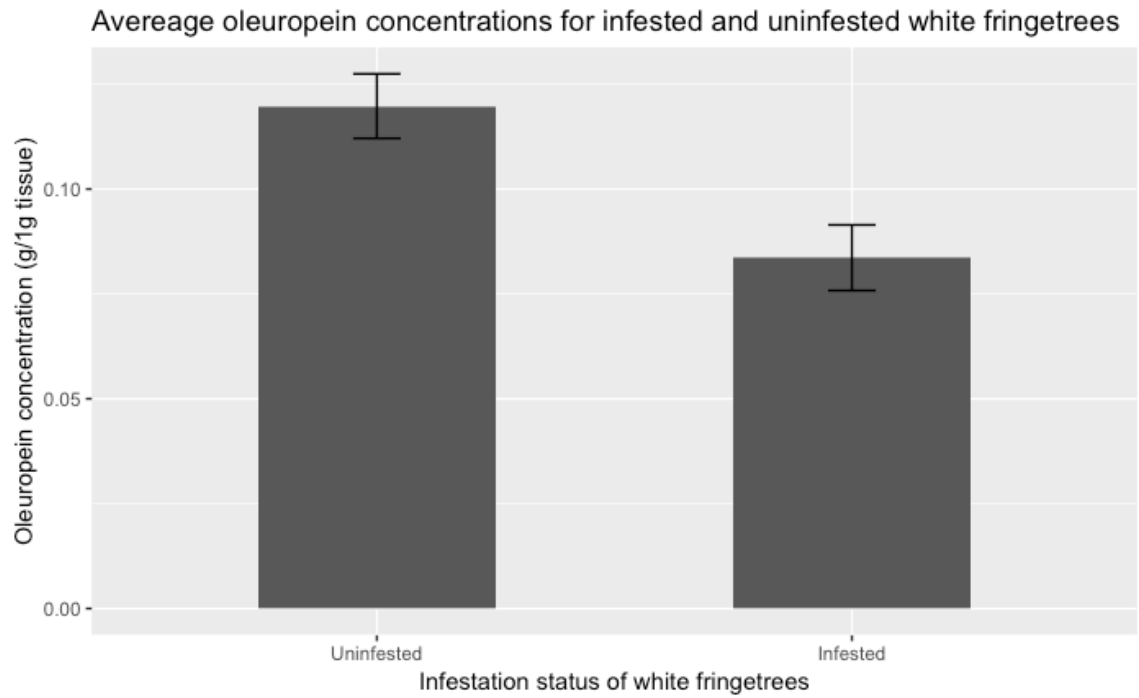


Figure 2.8. Average oleuropein concentrations between uninfested (0.12 g/sample) and infested (0.08 g/sample) white fringetrees (*Chionanthus virginicus*) with standard error bars.

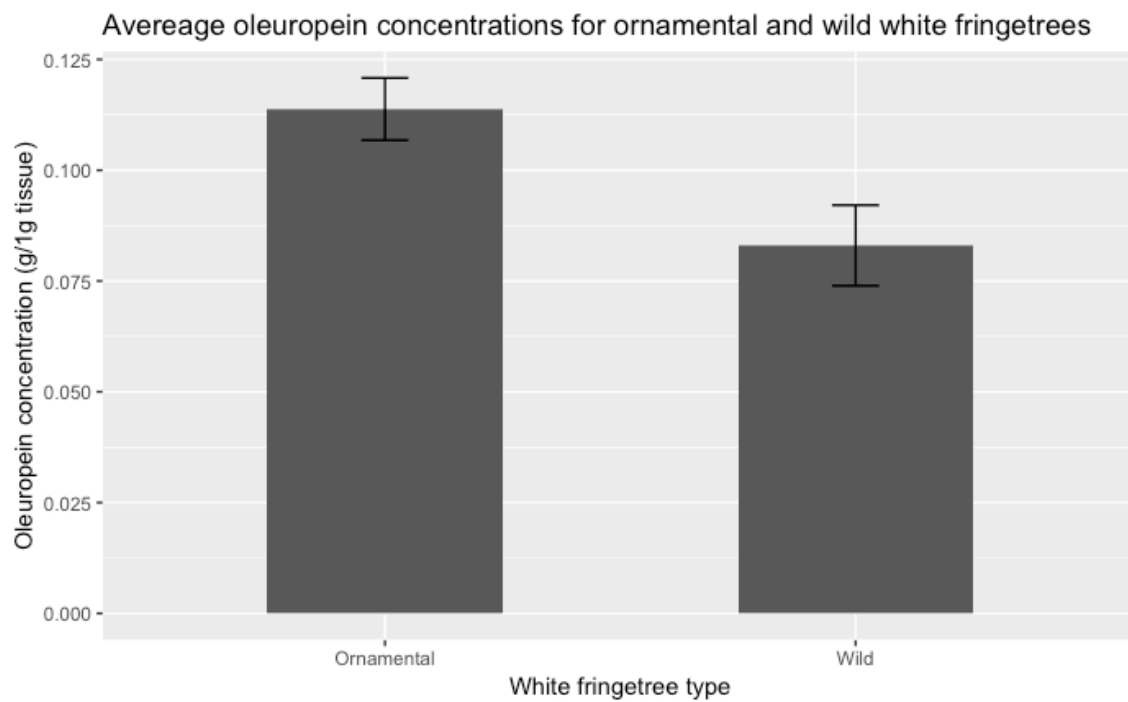


Figure 2.9. Average oleuropein concentrations between ornamental (0.11 g/sample) and wild (0.08 g/sample) white fringetrees (*Chionanthus virginicus*) with standard error bars.

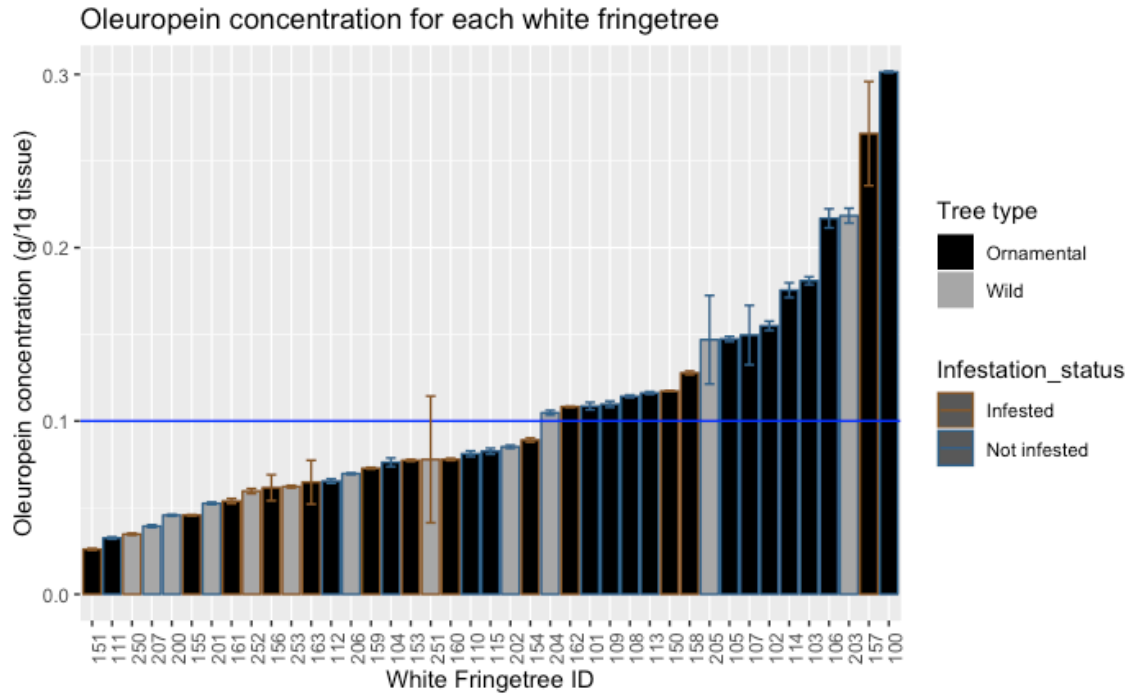


Figure 2.10. Oleuropein concentration in grams per one gram of sampled bark tissue for each white fringetree (*Chionanthus virginicus*) with standard error bars, overall mean gallery width line (0.10g/tissue), tree type, and infestation status.

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